Proposal for Research with Mass DPH and BU

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Attn: Julie Nassif
Lab Director
Massachusettes Department of Public Health

Keri LaBelle
Thesis work in correspondence with:
Boston University School of Medicine
Division of Graduate Medical Sciences
Biomedical Forensic Sciences Program
klabelle@bu.edu

Adam Hall, MS., D-ABC
Instructor of Forensic Chemistry
Chemistry-Trace Analysis Track Advisor
Boston University School of Medicine
Division of Graduate Medical Sciences
Biomedical Forensic Sciences Program
adamhall@bu.edu

Michael Lawler
Senior Forensic Chemist
Massachusetts Department of Public Health
Drug Evidence Lab
michael.lawler@state.ma.us

Summary/ Purpose of this proposal

1. **To Familiarize Reader on**
   - Illicit uses of *Salvia divinorum* and its active hallucinogenic compound Salvinorin A.
   - Salvinorin A analogues and recently published literature on their potential as substances of abuse.
   - Current and proposed control statuses of *Salvia divinorum* in the U.S.

2. **To Suggest for Research**
   - Explore existing methods of forensic identification of *Salvia divinorum*
   - Develop protocol for the identification of *Salvia divinorum*, Salvinorin A, and related Salvinorin A analogues
   - Validate protocol and instrumental methods in accordance with SWGDRUG guidelines

3. **To request permission to work with the Massachusetts Department of Public Health in their drug evidence section for partial completion of a Master's Thesis in Biomedical Forensic Sciences, to be obtained through Boston University.**

Salvia divinorum, a perennial herb from the Mint family originating from the Oaxaca, Mexico region, has been gaining popularity in the U.S. in the past few decades as a psychoactive plant material with hallucinogenic effects similar to those of other scheduled hallucinogenic substances. It exhibits a square stem reaching 0.5-1.5m and elliptical leaves that grow opposite one another, with flowering a rarity. In the few times the plant does bloom, it produces purple calyces and white flowers. However, there is a lack of outstanding characteristics separating it from other plants in the mint family. Forensically, visual identification of the plant material is not possible; even a skilled botanist may not be able to identify the plant material. As a result, forensic identification of *S. divinorum* relies on the identification of Salvinorin A, the active psychoactive compound in the plant. Salvinorin A (Sal A), is a neoclerodane diterpene, for which *S. divinorum* is the only known natural source. Studies have shown Sal A is a potent and selective kappa opioid receptor (KOR) agonist, unlike other schedule I hallucinogens which are primarily serotonin 2A receptor agonists.

Although other compounds have been isolated from *S. divinorum*, only Sal A has been found to be psychoactive. However, Sal A analogues have triggered the interests of *S. divinorum* users, as they are currently being synthesized and tested by researchers for potential therapeutic uses. Regardless of the route of ingestion, the effects of Sal A do not last longer than one hour, leaving current users searching to obtain a more potent and longer lasting high. Salvinorin B is a potential Sal A metabolite resulting from ester hydrolysis, which has been identified as a relatively inactive KOR agonist. Previous research by Béguin et al discovered that the most useful Sal A analogues would be those modified at the C2 carbon of Salvinorin B, specifically 2-Methoxymethyl-Salvinorin B (MOM), N-methylacetamide-Salvinorin B (NMA), and N-methylpropionamide Salvinorin B (MPA). Also of concern is 2-Ethoxymethyl Salvinorin B. These analogues are synthesized from Sal A, but were found to have more "desirable" characteristics, such as higher affinities for the KOR and longer durations of activation.

The potential for abuse of *S. divinorum* is high amongst teenagers and young adults (according to a National Survey on Drug Use and Health Report published by SAMHSA in February 2008), and as such the plant and its active compound have been repeatedly under consideration in numerous jurisdictions for regulation. Currently, neither *S. divinorum* nor its active compound Sal A are federally regulated under the Controlled Substances Act,

although they are listed on the "Drugs and Chemicals of Concern" list published online. As of 2009, fourteen U.S. states have placed *S. divinorum* onto controlled substance schedules. In addition, four states have enacted legislation controlling the sale and distribution of *S. divinorum* and many more have pending legislation, including Massachusetts. Throughout the world, fourteen countries have enacted laws regulating *S. divinorum* and Sal A.

Regulation of *S. divinorum* is necessary as it continues to grow as a popular recreational drug. Unfortunately, many of the quickly enacted legislation against *S. divinorum* are vague and incomplete. States enacting the earliest legislation made the plant itself illegal, and did not directly include the psychoactive compound Sal A, making these laws unenforceable for a number of reasons. A look at West Virginia's House bill on the "unlawful production, manufacture, or possession of *Salvia divinorum*" shows the inadequacy of laws only addressing the plant material, as it reads:

> (b) It is unlawful for any person to knowingly or intentionally manufacture or possess an extract, compound, concentrate, or other processed substance intended for human consumption which contains Salvia divinorum.
> (http://www.legis.state.wv.us/Bill_Status/bills_text.cfm?billdoc=HB4018%20SUB%20ENR.htm&yr=2010&sesstype=RS&i=4018)

Laws such as this are easily beat by users who can extract the plant oils and distribute them onto different substrates (such as other vegetation or sugar cubes) since the active hallucinogen Sal A is not mentioned. In contrast, the Illinois law regulating *S. divinorum* is all encompassing, reading:

> 10.5 Salvia divinorum (meaning all parts of the plant presently classified botanically as Salvia divinorum, whether grown or not, the seeds thereof, any extract from any part of the plant, and every compound, manufacture, salts, isomers, and salts of isomers whenever the existence of such salts, isomers and salts of isomers is possible within the specific chemical designation, derivative mixture, or preparation of that plant, its seeds or extracts).
> (http://www.ilga.gov/legislation/ilcs/documents/072005700K204.html)

Under this law, it could be interpreted that chlorophyll or other natural compounds of vegetative materials are regulated, when it is obvious that they are not. Even when plant material is collected as evidence, visual identification remains impractical from a forensic standpoint. As previously stated, the botanical features of *S. divinorum* are far from unique, and macroscopic or microscopic analyses could never be used as credible methods of identification as it is with marijuana.

New legislation attempting regulation of the distribution, possession, or abuse of S. *divinorum* must be clear and effective in addressing the active compound Sal A, along with any analogues deemed as potential substances of abuse. Proposed legislature in Massachusettes could place Salvia divinorum, Sal A, Salvinorin B, and 2 methoxy-methyl Salvinorin B on the Class C list, with a revision possible to include 2-ethoxymethyl Salvinorin B, (While Salvinorin B is not proven a hallucinogenic compound, its potential as an immediate precursor for synthesizing other hallucinogenic analogues makes it a compound of concern). Under Massachusetts general laws section 32B on Class C controlled substances, salts and isomers of controlled substances are held accountable, meaning that Sal A and its analogues would be regulated accordingly. Current drugs of abuse on this list include mescaline, peyote, and other psychoactive agents. Many websites suggest current hallucinogenic users are more likely to experiment with *S. divinorum*.

The forensic science community lacks a validated standard protocol for identifying *S. divinorum* and Sal A. A number of studies have provided guidelines for the extraction, purification, and analysis Sal A, however most were carried out in academic settings. As a result, the majority of published research on this subject uses highly specialized instrumentation, the majority of which are not cost effective for drug evidence laboratories in Massachusetts. Should legislature pass in Massachusetts, S. divinorum, Sal A, and the analogues of concern will be regulated as class C substances, resulting in an influx of evidence submissions which drug labs would not currently be able to accurately and quickly identify.

Visual examination of *S. divinorum*, whether fresh/dry leaves or whole plants, is not a useful identification method, as it has no distinct or unique botanical features. Commercially available microchemical tests have been explored as a potential preliminary test, but with little success. While they can differentiate *S. divinorum* from marijuana (Sal A vs THC, respectively), no distinctive color is viewed in the presence of *S. divinorum*. A botanical analysis of the plant material reveals little forensic information. The forensic identification of *S. divinorum* relies heavily upon instrument analysis of Sal A, using UV/Vis, FTIR, and GC/MS. UV/Vis has been documented as a successful screening tool with Sal A readily absorbs light at 210nm. However, extracting enough Sal A from plant material to gain a positive result can be complicated by plant pigments, and de-pigmentation attempts can limit the amounts of Sal A obtained. FTIR can provide a second method of screening for

S. divinorum but was found to be a time consuming process, requiring de-pigmentation and recrystallization steps. The preferred confirmatory test for *S. divinorum* was adopted from the method published by Giroud et. Al. in 2000 for evaluating unknown plant material via GC/MS. (Girouda, C, et. al. *Salvia divinorum*: a hallucinogenic mint which might become a new recreational drug in Switzerland. Forensic Science International, 122 (2000) 143-150)

While the forensic science community has adapted its current protocols from a number of studies, a validated protocol for *S. divinorum* must be developed in accordance with SWGDRUG guidelines. SWGDRUG requires the use of multiple uncorrelated techniques, so validating a single analytical method would not be entirely helpful. Much like the SWGDRUG recommendations for cannabis, without sufficient observable macroscopic and microscopic botanical data, the active compound (in this case Sal A) must be detected in plant material suspected of being *S. divinorum*, since visual identification is not feasible. This is useful in ruling out other Salvia species, none of which have been found to contain Sal A. Additionally, if extracts of Sal A are spiked onto other unrecognizable plant material, the sample would still fall under regulation and be deemed a controlled substance.

SWGDRUG supplemental document SD-2 outlines suggestions for quality assurance/validation of analytical methods, including: demonstrate the reproducibility of the instrument by running a reference material a minimum of 10 times, and determine the limit of detection below which no data will be accepted, determine the lowest concentration that has an acceptable level of uncertainty. Additionally, any interfering compounds must be determined and eliminate the possibility of a false positive. In the case of *S. divinorum*, a protocol must be able to identify the active chemicals of concern, including Sal A analogues which are also included in the bill. More research must be performed to make sure that Sal A can be differentiated successfully from its analogues.

The purpose of this research will be to build upon previous studies to establish a laboratory protocol for the analysis of the vegetative plant material *Salvia divinorum*. Considerations for this protocol include identifying the components of *S. divinorum* to be regulated by the proposed legislation: Sal A, 2 methoxymethyl Salvinorin B, and Salvinorin B, with considerations also for 2 ethoxymethyl Salvinorin B. This will be done by improving and validating current analysis methods in accordance with SWGDRUG guidelines in hopes of establishing a usable protocol in light of proposed legislature regulating *S. divinorum* in Massachusetts.